



**CLAIMS FOR APPLICATION SERIAL NO. 08/903,944**

**Clean Version of Amended Claims**

6. (Four Times Amended) A method for producing transgenic poinsettia plants, comprising:
- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
  - (c)
    - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* ~~containing the vector or vectors or by microprojectile-mediated delivery of the vector~~ into the callus;
  - (d) culturing said transformed embryogenic callus on selection medium;
  - (e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (f) culturing said transgenic embryos on maturation medium; and
  - (g) recovering transgenic plants from said transgenic embryos.
39. (Five Times Amended) A method for producing transgenic poinsettia plants, comprising:
- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;

- (b) subculturing embryogenic callus produced on said callus induction medium to liquid  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  containing embryo induction medium;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) subculturing embryos produced on said embryo induction medium to maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) subculturing epidermal callus produced on said callus induction medium to embryo induction medium to form embryogenic callus;
- (h)
  - (i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;

  - (i) culturing said transformed embryogenic callus on selection medium;
  - (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (k) culturing said transformed embryos on maturation medium; and
  - (l) recovering transgenic plants from said transgenic embryos.

102. (Three Times Amended) A method for producing transgenic poinsettia plants comprising the steps of:

- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;

- (b) subculturing embryogenic callus to embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus containing embryos;
- (c)
  - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

wherein the vector or vectors of (c)(i) and (c)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;
- (d) culturing said transformed embryogenic callus on selection medium;
- (e) culturing said embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (f) culturing said transgenic embryos on maturation medium; and
- (g) recovering transgenic plants from said transgenic embryos.

103. (Twice Amended) A method for producing transgenic poinsettia plants comprising the steps of:

- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
- (b) subculturing embryogenic callus produced on said callus induction medium to liquid embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$ ;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) subculturing embryos produced on said embryo induction medium to maturation medium;
- (f) culturing said embryos on callus induction medium;

- (g) subculturing embryogenic callus produced on said callus induction medium to embryo induction medium to form embryogenic callus containing embryos;
  - (h)
    - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;wherein the vector or vectors of (h)(i) and (h)(ii) are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;
  - (i) culturing said transformed embryogenic callus on selection medium;
  - (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (k) culturing said transformed embryos on maturation medium; and
  - (l) recovering transgenic plants from said transgenic embryos.
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